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With international search report.

(54) Title: RESTORATION OF IMMUNOCOMPETENCY TO T HELPER CELLS IN HIV INFECTED PATIENTS

(57) Abstract

This invention relates to the use of antagonists to IL-10 such as anti-IL-10 antibodies for pharmaceutical administration to patients infected with an immunodefenciency virus. The administration of these antibodies restores the ability of T helper cells to produce IL-2.

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RESTORATION OF IMMUNOCOMPETENCY TO T HELPER CELLS IN HIV INFECTED PATIENTS

BACKGROUND OF THE INVENTION

Field of the invention.

This invention relates to the use of antagonists against IL-10 for pharmaceutical administration to patients infected with a immunodeficiency virus. The administration of these antagonists restores the ability of T helper cells to produce IL-2.

SUMMARY OF THE INVENTION

This invention provides methods of increasing the level of IL-2 produced by T helper cells in a patient infected with a human immunodeficiency virus. The method comprises administering an amount of an antagonist of interleukin 10 wherein said amount is effective to increase the patient's T helper cell production of IL-2. The antagonists are preferably administered intravenously. A preferred antagonist is an antibody specific for binding to IL-10. The antibodies can be chimeric, recombinant, polyclonal or monoclonal. Autologous antibodies, human or humanized antibodies are preferred for safety when human patients are being treated. The preferred single dosage of antibodies is 1-10 mg/kg body weight per antibody. Alternatively the amount of the antibody administered in a single dose is about 10 to about 100 μg per milliliter of patient sera.

DETAILED DESCRIPTION

This invention provides an effective means for increasing production of IL-2 in T helper cells when said levels are being inhibited by excessive levels of IL-10 attendant an infection of lentiviruses known as the human immunodeficiency virus [HIV].

This chronic and often fatal viral infection is typified by an imbalance in T helper cellular responses. The result of this imbalance is an inhibition of IL-2 production by nonvirally infected T helper cells due to excessive levels of IL-10. The IL-10 is produced by a number of different cells including a subset of the T helper cells.

The maintenance of IL-2 production is beneficial to HIV infected patients. IL-2 is responsible for T cell proliferation and is a key indicator of the status of the immune system.

The reduction of IL-2 production can be halted by administration of adequate amounts of antagonists specific for IL-10. Antagonists of IL-10 can be made by mutating the amino acid sequence of IL-10 using standard mutagenesis methods. Such methods include the use of M13 vectors to introduce single site mutations, to delete random amino acids from IL-10 or to add amino acids. The resulting muteins are then tested in standard assays for the ability to compete with nonmutated IL-10. Suitable assays are described below and include *in vitro* cell assays where IL-2 dependent proliferation is initiated by the presence of exogenous IL-10. This strategy have been used to characterize the functional domains of numerous proteins such as thrombomodulin, human growth factor and tissue plasminogen activator.

Alternatively, the antagonist can be an antibody specific for binding to IL-10 [α IL-10] and which interferes with its binding to the T-helper receptor. α IL-10 is produced in a variety of conventional ways. A general review of antibody production can be found in Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Pubs., N.Y. (1988) or in Colligan *et al.* Eds., 1991 and Suppl. *Current Protocols in Immunology,* Green Wiley, NY, NY. Antibodies can be a polyclonal mixture or monoclonal. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources. Antibodies also include the immunoreactive portions of intact immunoglobulins.

In brief, methods to obtain α IL-10 antibodies involve administering an amount of antigen sufficient to induce a humoral response in a mammal. The antibodies are either collected from the mammal's sera or lymphocytes removed, immortalized and those cell

clones secreting the desired antibodies isolated and cultured for harvest of the desired antibodies. Antibodies against IL-10 are described by Mosmann *et al.*, 1990 *J. Immunol.* 145:2938.

These methods require adequate sources of IL-10 as antigens. The antigens can either be intact IL-10 or immunoreactive peptides. Recombinant expression of IL-10 is a convenient means for obtaining IL-10 for use as antigens. For a general review of the applicable recombinant technology see Sambrook, *et al., Molecular Cloning - A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1989. Specific techniques for expressing and purifying IL-10 are known. Expression of IL-10 is described in PCT/US/03554 (WO/91/00349) and in Malefyt, *et al.*, 1992, *Curr. Opin. Immunology,* 4:314-320. Alternatively, peptide synthesis may be used to obtain intact or immunoreactive portions of IL-10.

The antibodies for use in this invention are preferably autologous for the patient thereby minimizing further immunological problems. Immunodeficient individuals will tend to be less reactive to non-self antibodies, and thus non-self antibodies derived from cells of the same species are also useful. Antibodies of different species are useful but means to control possible adverse immunoreactions must be undertaken. For example, humanized rat antibodies can minimize immune responses in human patients.

The antibodies for use in this invention are typically neutralizing antibodies and will preferably have binding constants which are greater than or approximates the affinity of IL-10 for its natural receptor. Antibodies having a binding constant 100-fold less than these cytokines for their corresponding receptors are less preferred. Binding comparisons are carried out using standard equilibrium methods. The basic technology is described in Chapter 25 of Vol. 1: Immunochemistry, Ed. D.M. Weir, 4th Ed. 1986, Blackwell Scientific Publ. 25. 1-25.30. Alternatively, one can use an assay for determining the molar excess of antibody which neutralizes a defined amount of IL-10 in a standard *in vitro* bioassay. Examples of such assays are found in Mosmann and Fong, 1989, *J. Immunol. Methods, 116* : 151 (IL-4) and Fiorentino *et al.*,

1989, *J. Exp. Med. 170* : 2081. A reasonable range are those antibodies which neutralize a given amount of IL-10 in a 10 to 1,000 fold excess.

The means of administration of the antagonists, e.g. α IL-10 are typically parenteral, preferably intravenous. The antagonists are infused into the patient using standard intravenous techniques. The antagonists are first suspended into a sterile, physiologically-compatible media, such as phosphate buffered saline. Pharmaceutically acceptable excipients such as lecithin, glucose, dextrose, antibiotics may also be included with the antagonists.

When the antagonists are antibodies, they are administered in an amount which provides circulating levels of α IL-10 at about 1 to 150 μg/ml and preferably 10 to 100 μg/ml of sera for each antibody. The antibodies have a 2-7 day half-life and repeated administration is necessary when levels of α IL-10 are below these levels. Total amount of α IL-10 applied per administration are between 1 and 10 mg/kg of body weight for each antibody.

The method will increase IL-2 production and it is preferred that said levels approach or exceed 100% of normal. Increases of greater than 50% of the IL-2 production before treatment are considered good. Treatment can be terminated when the T helper cells are producing levels of IL-2 at 10 to 100% of normal when measured by any number of conventional assays.

These conventional assays fall into two categories. The first category are bioassays that measure IL-2 dependent proliferation of any of several immortal cell lines which proliferate in the presence of IL-2. An example of such a cell line is CTLL. Cell division is measured by radiolabelled thymidine uptake. The second category are functional assays and involve the use of immunoassays directly measuring IL-2 such as ELISA. A general overview of this art can be found in Mosmann and Fong, 1989, Specific assays for cytokine production by T cells, *J. Immunol. Meth. 116* : 151-158.

Conventional antiviral therapies such as AZT, may also be used in conjunction with this invention.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be recognized that certain changes and modifications may be practiced within the scope of the claims.

WHAT IS CLAIMED IS:

1. A method of increasing the level of T helper cell production of interleukin 2 in a patient infected with a human immunodeficiency virus said method comprising administering an amount of an antagonist of interleukin 10 (IL-10) effective to increase the patient's T helper cell production of IL-2.

2. A method for the manufacture of a pharmaceutical composition for increasing the level of T helper cell production of interleukin 2 in a patient infected with a human immunodeficiency virus comprising admixing an antagonist to IL-10 with a pharmaceutically acceptable carrier.

3. The method of any one of claims 1-2 in which the antagonist is an antibody to IL-10.

4. A pharmaceutical composition for increasing the level of T helper cell production of interleukin 2 in a patient infected with a human immunodeficiency virus comprising an antagonist to IL-10 and a pharmaceutically acceptable carrier.

5. The pharmaceutical composition of claim 4 in which the antagonist is an antibody to IL-10.

6. The use of an antagonist to IL-10 for increasing the level of T helper cell production of interleukin 2 in a patient infected with a human immunodeficiency virus.

7. The use of an antagonist to IL-10 for the manufacture of a medicament for increasing the level of T helper cell production of interleukin 2 in a patient infected with a human immunodeficiency virus.

8. The use of either claim 6 or 7 in which the antagonist to IL-10 is an antibody to IL-10.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/08562

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K39/395 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	EP,A,0 541 214 (SCHERING CORPORATION) 12 May 1993 see page 44, line 51 - page 53, line 19	1-8
X	EP,A,0 405 980 (SCHERING CORPORATION) 2 January 1991 cited in the application see page 10, line 8 - line 28	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
6 December 1993	22 -12- 1993

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08562

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 1,3,6,8 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort jusufying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P. /US 93/08562

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0541214	12-05-93	AU-A-	2441192	02-03-93
		WO-A-	9302693	18-02-93
EP-A-0405980	02-01-91	AU-B-	635058	11-03-93
		AU-A-	6077090	17-01-91
		CA-A-	2062763	29-12-90
		CN-A-	1051393	15-05-91
		EP-A-	0567450	03-11-93
		JP-T-	4502560	14-05-92
		WO-A-	9100349	10-01-91
		US-A-	5231012	27-07-93